Attorney Docket No.: 1201-73

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT OPERATION

In re Application of:	Schneider, et al.		
Serial No.:	09/401,838	Art Unit:	1616
Filed:	September 22, 1999	Examiner:	M. Hartley
For:	Ultrasound Contrast Agents And Methods Of Making And Using Them		

Assistant Commissioner of Patents
Washington, DC 20231

July 9th, 2002

Declaration Of Michel Schneider, Ph.D.

I, Michel Schneider, declare as follows:

1. I reside in Troinex, Switzerland.

2. I am a co-inventor of U.S.S.N. 09/401,838 ("the '838 application"), as well as its' parent U.S. Patent No. 5,277,928 and U.S. Patent No. 5,413,774, the specifications of which are included in the '838 application.

3. I have previously planned and supervised the experiments which led to the data found in Table 4 of the '838 application. These experiments establish that the phospholipid stabilized microbubbles of the '838 application are unexpectedly superior to the use of air in the same phospholipid stabilized microbubbles. The phospholipid stabilized microbubbles of the '838 application contain well known representative freon gases.

4. To further confirm the unexpectedly superior properties of the phospholipid stabilized microbubbles of the '838 application to the closest prior art gas of air, I have performed additional experiments comparing the *in vivo* characteristics and persistence of phospholipid stabilized microbubbles containing air, with phospholipid stabilized microbubbles

containing well known freon gases such as CF_4, C_2F_6, C_2ClF_5, C_3F_8, and C_4F_8 (cyclic). The fluorinated carbon-containing gases used here are repr sentative of the family of freon gases. I believe that this experiment is a more than fair comparison. We used the phospholipid stabilized microbubbles of my invention with the closest prior art gas air, instead of the dramatically inferior phospholipid containing agents of the prior art.

5. Phospholipid stabilized microbubbles were prepared from a solution containing distilled water, 1% dipalmitoylphosphatidyl glycerol, 3% Pluronic F68 and 3.6% glycerol which was heated at about 80°C. The clear homogeneous solution was then cooled to room temperature and as explained in the next paragraph, bubble suspensions were produced with air, CF_4, C_2F_6, C_3F_8, and C_4F_8 (cyclic).

6. The bubble suspensions were obtained using two syringes connected via a three way valve. One syringe was filled with 6 ml of the phospholipid solution with 1 ml being discharged through the valve while the other was filled with 1 ml of the desired gas. The three way valve was filled with the phospholipid solution before it was connected to the gas-containing syringe. By alternatively operating the two pistons, the phospholipid solution was transferred back and forth between the two syringes (25 times in each direction) forming a milky suspension.

7. Four different preparations of each suspension were each injected into a different New Zealand rabbit and *in vivo* echogenicity was assessed. Test samples (0.05 ml test sample/kg body weight) were injected intravenously in the ear vein of the 3-4 kg rabbits which had been anesthetized with Narcoren (pentobarbital). Ultrasound imaging of the heart (short axis view) was performed using an Acuson 128 XP/10 equipped with a linear probe (7 MHz). Power was set at -9dB. The images were recorded and the intensities of the area of interest (AOI) on the

video images were analyzed as a function of time. The peak intensity, the half life and the area under the curve were determined for each sample. Measurements were performed in the right as well as the left ventricle. Additionally, "persistence" was determined as half of the time elapsed between injection of the sample until the signal returned to baseline.

8. From my experience, I note that the key to determining the usefulness and efficacy of an ultrasound contrast agent when imaging the heart are the results obtained for the left ventricle of the heart. As there are virtually no pathologies associated only with the right ventricle of the heart, the ability to enhance the left ventricle is the only useful clinical parameter for an ultrasound contrast agent since it shows the ability of the bubbles to cross the lungs and thereafter reach the systemic circulation.

9. Thus, a statistical analysis of the raw data was performed on the left ventricle, and the mean measurements and the standard errors of the mean ("SEM") are set forth in Table 1 attached hereto. Whether contrast enhancement is measured by half life, area under the curve or persistence value, the phospholipid stabilized microbubbles containing freon gases all proved significantly more effective in the left ventricle than phospholipid stabilized microbubbles containing air. Specifically, the half life, area under the curve and persistence measurements for the phospholipid stabilized microbubbles containing freon gases range from nearly twice to more than seven times greater than those measurements for phospholipid stabilized microbubbles containing air.

10. Thus, these results confirm that the phospholipid stabilized microbubbles of the '838 application which contain freon gases are, surprisingly, significantly more effective than the stabilized microbubbles with the closest prior art gas - air.

All statements made of my own knowledge are true and all statements made on information and belief are believed to be true. I make this declaration understanding that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the applications or any patent issuing thereon.

Respectfully submitted,

Michel Schneider, Ph.D.

Dated: July 9th, 2002